Determining the Effects of the Coagulant, Aluminum Sulfate, on the Adsorption of Microcystin-LR on Powdered Activated Carbon

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Abstract

Based on climate change projections, the occurrence and intensity of cyanobacterial blooms is expected to increase; also it is more likely that these blooms will produce multiple cyanotoxins in drinking water supplies. Therefore, the drinking water industry needs to understand how to most effectively remove a range of cyanotoxins from the water column. The most common cyanotoxin found in the United States is microcystin, and the most common isoform of microcystin is microcystin-LR (MCLR). Microcystin-LR can be found in both intracellular and extracellular forms in drinking water. Typical water treatment processes, such as coagulation/flocculation, filtration, and sedimentation, are ineffective against extracellular toxins. Powdered activated carbon (PAC) has been shown to be effective against extracellular toxins. In this study, the effectiveness of PAC application under typical coagulation/flocculation processes and conditions was tested. Batch tests were performed to determine how adsorbent dose and presence of coagulant (specifically, aluminum sulfate) impact the adsorption process, and therefore, MCLR removal. It was decided that the coagulant and PAC should be added simultaneously to reduce the effect of natural organic matter adsorption competition. As expected, the addition of the alum resulted in a decrease in the amount of MCLR adsorbed to the PAC; however, overall MCLR removal increased when alum was added. It is hypothesized that this increase is due to adsorption to aluminum hydroxide particle, but more research is needed to confirm this. Results of this test will be used to develop guidelines and best practices to be used by utilities to optimize cyanotoxin removal.

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Chapter 1. Introduction

1.1 Background

Microcystin is a cyclic heptapeptide heptatoxin that is formed from the cyanobacteria of genera Microcystis, Anabaerna, Nostoc and Oscillatoria (Bourne et al., 2001). These algal blooms are caused by the combination of excess nitrogen and phosphorus runoff into surface waters and the increase in temperatures of these waters, creating manmade eutrophication of many lakes and rivers worldwide.

Microcystin has only been an issue of concern recently. In 1996, about 50 people died in a hemodialysis facility in Brazil caused by microcystin contamination of the water (Yan et al., 2012). This caused the World Health Organization (WHO) to create a drinking water standard of 1 ppb for microcystin. Microcystins are heptatoxins and tumor-promoters; microcystin causes inhibition of proteins serine/threonine, phosphateases 1 and 2A (Imanishi et al., 2005). Microcystin affects livestock as well as humans, and a specific isoform, MCLR, may be a human carcinogen (Edwards et al., 2008). Due to concerns over the health effects on sensitive populations, the US EPA encouraged the state of Ohio to decrease the drinking water standard to 0.3 ppb for total microcystin (US EPA, 2016). Microcystin is on the Contaminant Candidate List, which means the federal government is considering making it a maximum contaminant level (Szlag et al. 2015).

According to Somdee et al. (2013), more than 70 isoforms of microcystin have been identified. They all consist of 7 amino acids; there are two L-amino acids, whose identities determine the identity of the microcystin isoform (cyanosite.bio.purdue.edu). Figure 1a depicts

the template of a microcystin isoform. The most studied and most commonly found isoform is MCLR. It is also one of the most toxic microcystin isoforms. The identities of the two variable L-amino acids for MCLR are leucine in the 2 position and arginine in the 4 position in Figure 1a. Figure 1b shows the structure of MCLR.

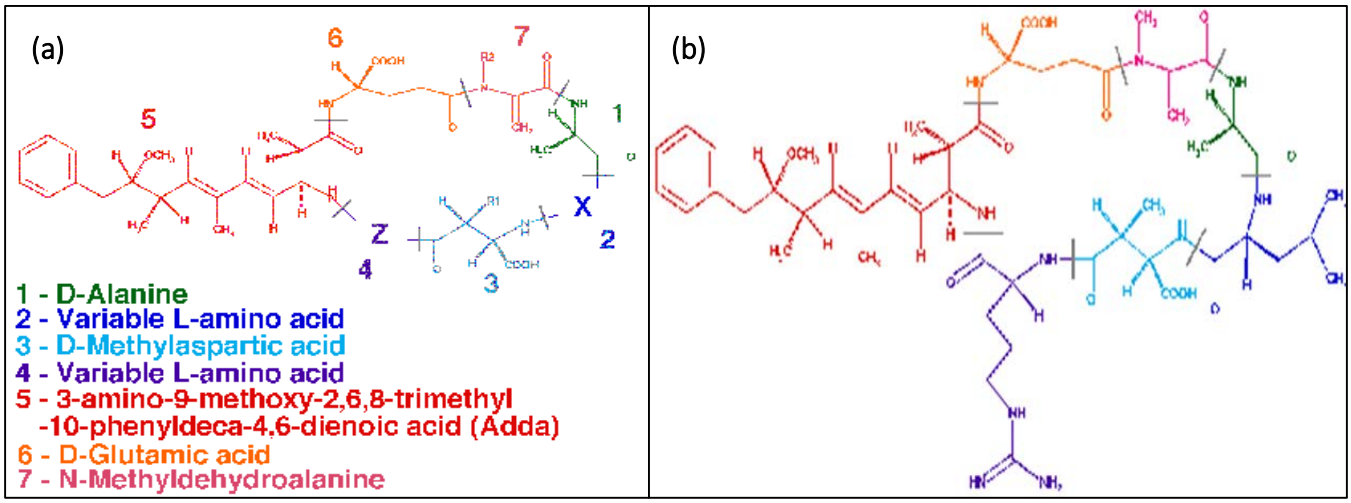

Figure 1. Chemical structure of microcystin. (a) General structure of any microcystin isoform (b) Structure of MCLR with the amino acids leucine and arginine. This is the most commonly measured isoform. Source: cyanosite.bio.purdue.edu

Because of its small cyclic structure, microcystin is chemically stable in water. This means that microcystin is recalcitrant to most drinking water treatment methods (Junfeng et al., 2009). Microcystin can be found in both intracellular and extracellular form; it is intracellular 95% of the time during the growth stage but when the cells die or the cell membrane ruptures, microcystin is released in its extracellular form (Szlag et al. 2015; US EPA, 2014). Variable microcystin-LR concentrations of 0.04 to 15,000 ppb have been found throughout the world (US EPA, 2015). While cyanobacterial cells (and therefore intracellular toxins) are easily removed by traditional water treatment processes such as coagulation/flocculation, sedimentation and filtration, extracellular toxins have only limited removal in these systems (Ho et al. 2011; US EPA, 2014; Ho et al. 2008). In addition, extracellular toxins are more likely to adsorb to colloidal

and suspended particles, such as natural organic material, in the water column, making them more difficult to remove (US EPA, 2014).

Adsorption by activated carbon, exclusion by membrane filtration, and chemical inactivation (such as ultraviolet, disinfectants and oxidants) have been shown to effectively remove extracellular toxins (US EPA, 2014; Westrick et al. 2010). Granular activated carbon (GAC) has been shown to be effective for removal of microcystin (primarily due to it being in intracellular form), but less effective at removing extracellular toxins, especially anatoxin-a and cylindrospermopsin, than powdered activated carbon (PAC) (US EPA, 2014; Westrick et al. 2010).

PAC and chlorination are the most commonly used treatment methods for extracellular toxin removal (Ho et al. 2008). PAC is an easy temporary treatment of seasonal contaminants such as cyanotoxins (Westrick et al. 2010). Many PAC adsorption studies have been conducted on microcystin isoforms (Ho et al. 2011). Based on these studies, it has been determined that PAC types with a high mesopore[1] capacity, mainly wood-based, are most effective for MCLR removal (Donati et al., 1994; Westrick et al. 2010; Huang et al., 2007; Lee and Walker, 2006).

Due to climate change, not only is the occurrence and intensity of cyanobacterial blooms expected to increase, but also it is more likely that multiple cyanotoxins will be present in drinking water supplies. Therefore, it is paramount that the industry understands how best to remove a range of cyanotoxins so treatment plants can make plans to deal with worsening algal blooms (Ho et al. 2011).

[1] Mesoporous carbon means that at least 50% of the total pore volume falls within the 20 to 500 angstrom range and no more than 25% of the pore volume is >500 angstroms (Peng et al. 2000).

1.2 Objectives

PAC has been shown to be effective at removing MCLR at high doses (Ho et al. 2011). However, this has not been tested under application in a traditional coagulation/flocculation process, which is typically when PAC is added. PAC is added prior to coagulation and removed in the settling tanks, or added in the settling tanks and removed via filtration (Ohio EPA, 2015). Since there is competition between natural organic matter and MCLR for adsorption sites on PAC, it was decided to add the PAC and the coagulant simultaneously to downplay this factor. The effects of the addition of the coagulant aluminum sulfate, or alum, on PAC dosage for removal of MCLR, under conditions found in a typical water treatment system, were investigated. The methodology, results and conclusions are presented below.

Chapter 2. Methodology

2.1 Overview

Sample water was taken from the Olentangy River after a heavy rain. The optimal alum dose of 40 mg/L was determined with a jar test. Two adsorption experiments were performed. One set was sample water and PAC to set a baseline for the percent MCLR removal for each PAC dose. The second set included an alum dose along with the sample water and PAC dose. Samples were filtered with a 0.7 µm glass fiber filter. MCLR measurements were made using an Enzyme-Linked Immunosorbent Assay (ELISA) plate kit.

2.2 Model Water and PAC Characteristics

Initially, an attempt was made to create model water based on that of Grand Lake St. Mary's. However, the model water was too homogeneous and did not coagulate in the same manner as natural water. Therefore, sample water from the Olentangy River was used. The water was spiked with 10 µg/L extracellular MCLR immediately prior to the commencement of the experiment.

The PAC used in this experiment was made by Ingevity. About two-thirds of the volume is mesoporous (66%), providing plenty of spaces for MCLR to adsorb to. See Table 1 for further information on the characteristics of the PAC.

Table 1. PAC characteristics

Brand	**Source Material**	**Activation Method**	**Iodine Number**	**Micropore Volume**	**Mesopore Volume**	**Macropore Volume**
Aqua Nuchar	Wood	Chemical	>900	32%	66%	2%

2.3 Adsorption Experiments

One liter aliquots of the sample water were dosed with 0, 5, 6, 8, 9 and 10 mg/L of wood-based PAC. The 0 mg/L dose is a control to separate the removal occurring because of PAC and that occurring through natural degradation and loss in the system. These doses were based on those used in literature. The lower doses were used as opposed to the higher ones seen in literature (25 and 100 mg/L) as these were shown to have higher adsorption rates for MCLR.

These experiments were set up in a similar manner as a jar test. The PAC and coagulant were added during rapid mix since natural organic matter (NOM) competition for adsorption sites is an issue if PAC is added prior to coagulant addition (Ohio EPA, 2015). The samples were mixed at a speed of 80 rpm for 20 seconds in order to assure maximum dispersion of the chemical(s). The speed was lowered to 10 rpm for 30 minutes to allow for flocculation and/or PAC to have adequate contact time with MCLR for adsorption. This was followed by a 30 minute sedimentation period.

The first set of these experiments was performed with just the PAC and sample water. This experiment was repeated but this time a 40 mg/L alum dose was added to each sample. The percent of MCLR removal was compared between the two sets of jar tests to see how PAC removal was affected by the addition of alum. 200 mL of each sample was filtered with a 0.7 micron glass fiber filter to remove the PAC and flocs from solution.

2.4 ELISA

A Beacon Analytical Systems microcystin plate kit was used. This ELISA plate follows a direct process. This process is detailed in Figure 2.

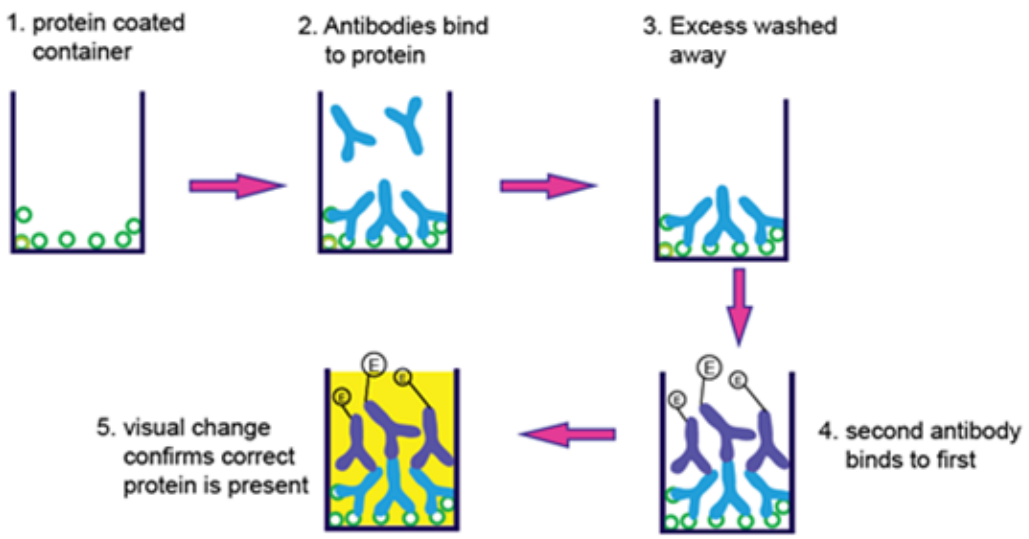

Figure 2. Diagram of a direct ELISA process. The second antibody contains a substrate that reacts and changes color. Source: http://nptel.ac.in/courses/122103039/module5/lec35/2.html

The wells in the plate are coated with a polyclonal antibody that binds with many microcystin isoforms as well as microcystin enzyme conjugates. First 50 µL of microcystin enzyme conjugate is added to each well. Then 50 µL of calibrators, control and samples are added to the appropriate wells. After incubating for 30 minutes and washing the wells five times, 100 µL of substrate (or second antibody) is added to the wells. This substrate binds only to the enzyme conjugate, not a microcystin isoform. Therefore, the more microcystin in the sample, the less the reaction with the substrate, and the lighter the final color of the well. After this addition the wells are incubated for 30 minutes and then 100 µL of stop solution (1 N hydrochloric acid) is added to each well. The detection limit was 0.005 ppb on the low end and 2 ppb at the high end. Another limitation to this method is that only total microcystin is measured, as opposed to individual isoforms.

The absorbance of the plates was then read at 450 nm with a Biotek plate reader. The data-reduction capabilities were utilized and a nonlinear regression (4-parameter) was used to produce a standard curve.

Chapter 3. Results and Discussion

3.1 Overall MCLR Removal

Surprisingly, when alum was added to the PAC the overall removal of MCLR was greater than that of PAC alone. Since coagulation/flocculation/sedimentation is not supposed to remove extracellular toxins (Ho et al., 2011), it was expected that the overall MCLR removal would decrease. But according to Figure 3, the overall removal increases with the addition of alum. Overall removal was calculated using Equation 1:

$$Removal = \frac{[MC]_0 - [MC]_d}{[MC]_0} \times 100\% \quad (1)$$

Where $[MC]_d$ is the microcystin concentration at PAC dose, d, and $[MC]_0$ is the microcystin concentration for the 0 mg/L PAC dose for the PAC only test.

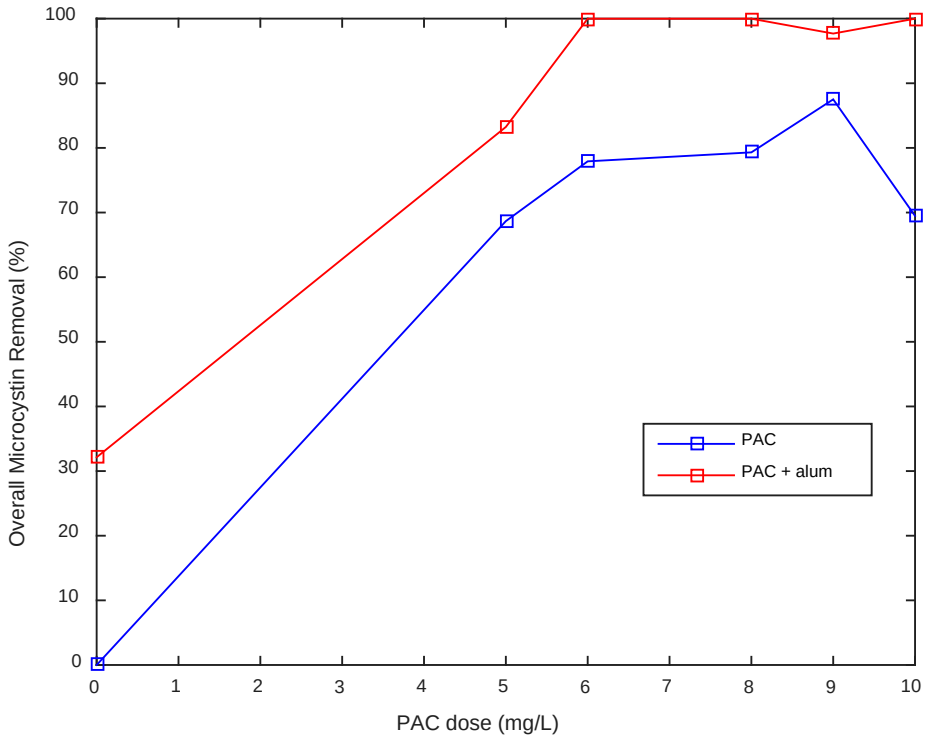

Figure 3. Comparison of removal efficiencies between that of PAC alone and that of PAC and alum at 40 mg/L. Removal was determined by subtracting the concentration of the control for the PAC only test from each sample.

Comparing microcystin removals between the controls (0 mg/L PAC dose) used in the two runs, it was determined that the removal due to alum was up to 32.14%. Alum reacts in the water to produce aluminum hydroxide according to the following reaction:

$$Al_2(SO_4)_3 \cdot 14.3H_2O + 6HCO_3^- \leftrightarrow 2Al(OH)_3 \downarrow + 3SO_4^{2-} + 6CO_2 + 14.3H_2O$$

As this reaction goes on, alkalinity is consumed and the pH drops. This was observed in the experiment as the pH changed from 7.98 to 7.16 after the addition of alum. The aluminum hydroxide particles may be acting as another substrate for the microcystin to adsorb to, increasing the overall removal of MCLR in the system.

Lee and Walker (2011) studied the adsorption of MCLR onto iron oxide. This is the first paper to study the interactions between MCLR and metal oxides and hydroxides. Based on their data, as pH decreases, the adsorption of MCLR on iron oxide increases. At a starting concentration of 50 ppb (5 times greater than that used in this experiment), about 17% of MCLR had adsorbed to the iron oxide at a pH of around 7.2. This pH is close to the ending pH after the addition of alum to the jar tests. Iron oxide is not the same as aluminum hydroxide, and it is not certain how exactly aluminum hydroxide will react with MCLR, but this data can serve as a basis for a hypothesis. Iron oxide only adsorbs about 17% of the MCLR at this pH but the different properties of aluminum hydroxide may allow it to adsorb twice as much (~32.14%). Based on Lee and Walker's (2011) data, it is plausible that aluminum hydroxide adsorption is accounting for the additional MCLR removal seen in the system.

3.2 MCLR Removal due to PAC

It was hypothesized that when alum was added to the system, MCLR removal due to PAC would decrease because the PAC would become incorporated into flocs before all the adsorption sites could be utilized by the microcystin. As can be seen in Figure 4, this did in fact occur.

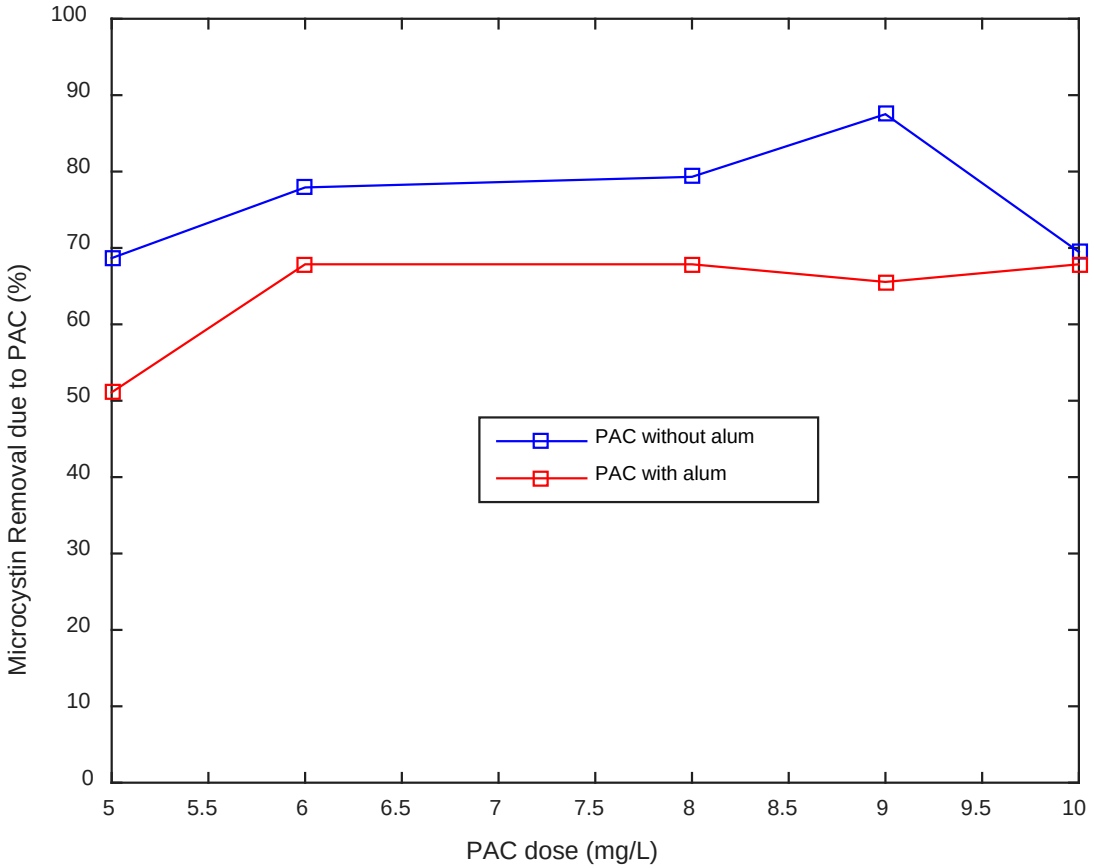

Figure 4. Removal efficiencies of only PAC after the removal due to alum is removed. Removal was determined by subtracting the removal due to alum (0 mg/L PAC dose in the PAC + alum test) from overall removal for each of the samples from that test.

To determine the optimum PAC dose for this system, the adsorption of MCLR per milligram of PAC added was calculated for each dose. These can be seen in Figure 5. These values were calculated following Equation 2.

$$adsorption = \frac{[MC]_0 - [MC]_d}{d} \quad (2)$$

Where $[MC]_d$ is the microcystin concentration (in µg/L) at PAC dose, d, (in mg/L) and $[MC]_0$ is the microcystin concentration for the 0 mg/L PAC dose for the PAC only test. For the PAC plus alum test, the values for $[MC]_0$ were corrected by the amount of microcystin removed by alum. Comparisons were then made between the adsorption between PAC without alum and PAC with alum.

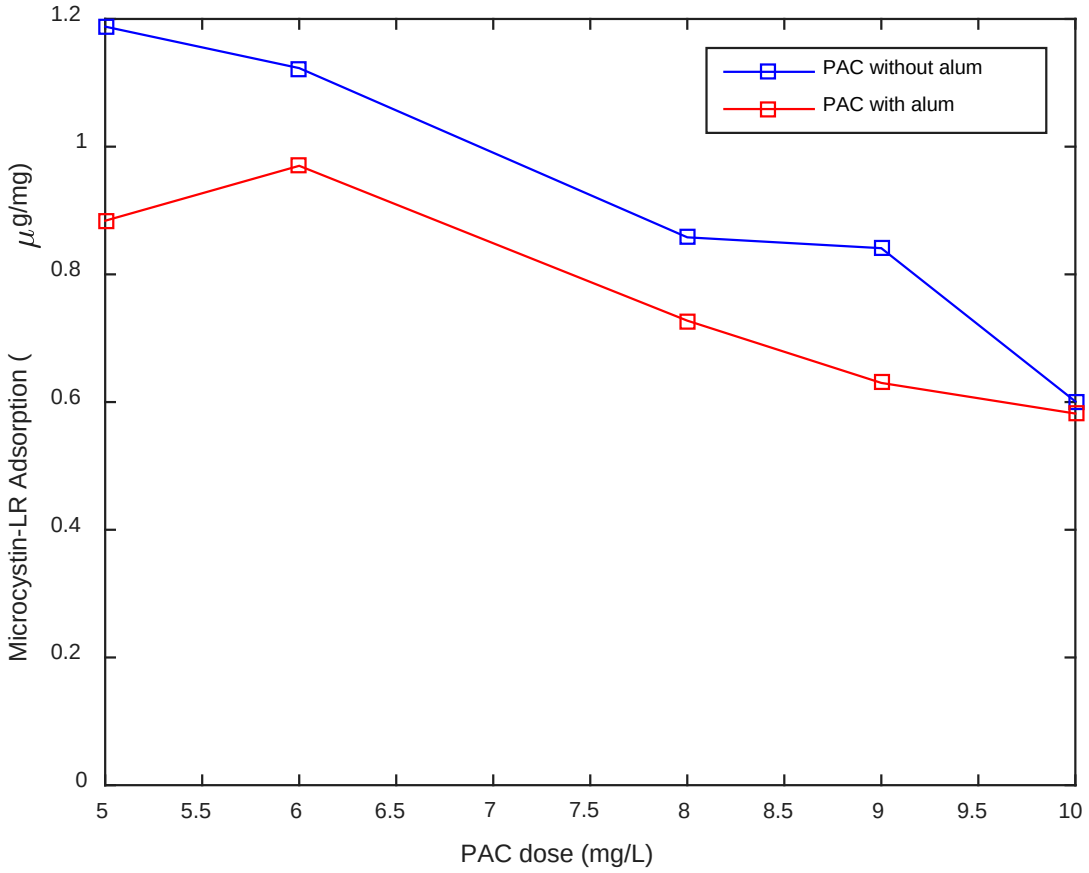

Figure 5. The amount of MCLR adsorbed per milligram of PAC added to the sample.

More MCLR is able to adsorb to the PAC without the addition of alum. When alum is added the most appropriate PAC dose for the system is 6 mg/L, while 5 mg/L is the most appropriate dose when alum is not added. Sorlini and Collivignarelli (2011) tested the removal efficiency of

MCLR by a mesoporous carbon under similar conditions. Based on results from their batch test, they had values of 1.1 – 1.2 µg MCLR/mg PAC for their adsorption when the system was dosed with 8 mg/L PAC and 10 ppb MCLR, which correspond well with the data in Figure 5. Ho et al. (2011) also performed a similar batch test and obtained a maximum adsorption value of 0.62 µg MCLR/mg PAC for their 5 mg/L dose. While this is close to the values of the data in Figure 5, it's not entirely the same. The difference between this experiment and that presented by Ho et al. (2011) is that they tested four microcystin isoforms (MCLR, MCYR, MCRR, and MCLA) at the same time, making a total microcystin concentration of 22 ppb. The difference in their adsorption values from those in Figure 5 can be accounted for by adsorption competition between the different isoforms.

There was a slight error that occurred with the 10 mg/L PAC dose. In the PAC without alum dataset, the 10 mg/L dose is about 20% lower in removal than the 9 mg/L dose. A contributing factor in the variance between 10 mg/L and the 9 mg/L dose may be attributed to the usage of two liter beaker with the 10 mg/L test and usage of a one liter beaker in all other tests. The size variance may have reduced the effectiveness of the mixing in the two liter beaker, thereby decreasing contact time with the PAC in that jar. However, when comparing the other doses, there is a pretty clear trend developed in Figures 4 and 5. Since there was not enough time to perform multiples, it cannot be determined if this is an inherent or systematic error. All other tests demonstrated consistency and determined to be reliable and trustworthy.

Chapter 4. Conclusions

For the conditions studied, the addition of alum does not inhibit the removal of microcystin during coagulation/flocculation, but it actually improves overall removal. However, the removal due solely to PAC decreases when alum is added. The decrease in removal due to PAC can be explained by incorporation into flocs. More research needs to be performed in this area to confirm that this is the mechanism at play. The loss of PAC due to flocculation needs to be quantified. This could be done by retaining the flocs of a jar test and measuring the amount of carbon in the floc. Comparisons could then be made between flocs from jar tests with PAC and from those without PAC.

This research has only scratched the surface on this subject as well. To determine if adsorption of MCLR on to aluminum hydroxide is the cause of the extra removal, more experiments need to be performed. First the amount of aluminum hydroxide that is produced during coagulation/flocculation needs to be measured to see if there is even enough aluminum hydroxide being produced to provide sufficient removal. Second, an adsorption isotherm needs to be made to determine how much microcystin can adsorb to aluminum hydroxide.

More research is needed to determine the interactions that occur during coagulation/flocculation with PAC that affect the removal of cyanotoxins. This research is important for water treatment plants (WTPs) since reducing PAC during seasonal algal blooms reduces chemical costs, which reduces utility costs for the residents of that WTP. This will become more pertinent as algal blooms will increase in intensity and occurrence.

Appendix A: Raw MCLR data

Table 2. Microcystin removal due solely to PAC. Raw data and removal efficiencies are included.

PAC Dose (mg/L)	[MCLR] for first[2] test (ppb)	% Removal for first test	[MCLR] for second test (ppb)	% Overall Removal for second test	% Removal due to Pac for second test
0	8.65	-	5.87	32.14	
5	2.71	68.67	1.45	83.24	51.10
6	1.91	77.92	below detect limit[3]	>99.99	>67.86
8	1.79	79.31	below detect limit	>99.99	>67.86
9	1.08	87.51	0.2	97.69	65.55
10	2.64	69.48	below detect limit	>99.99	>67.86

[2] First test refers to addition of solely PAC. Second test refers to addition of PAC + alum.
[3] Detection limit is 0.005 ppb for this system.

Appendix B: A note on cylindrospermopsin

This experiment was to be conducted with cylindrospermopsin (CYN), as it is a predominantly extracellular toxin and is increasingly found in drinking water supplies. However, issues were encountered with the analytical methods. A combination of solid phase extraction (SPE) and ultra-pressure liquid chromatography (UPLC) were to be used to measure the CYN concentration in the final samples. However, the detection limit on the Waters UPLC/PDA was very high (about 200 ppb) using both a methanol and acetonitrile gradient. Since the system would only be spiked at 10 ppb, the samples would need to be concentrated with SPE. However, the HLB cartridges used for SPE in the lab were not able to capture CYN; therefore, the samples could not be concentrated. Instead of using large amounts of CYN for the experiment (which is costly), MCLR was adopted as there were already established analytical methods for it in the lab.

References

Bourne, David G., Peter Riddles, Gary J. Jones, Wendy Smith, and Robert L. Blakeley. "Characterisation of a Gene Cluster Involved in Bacterial Degradation of the Cyanobacterial Toxin Microcystin LR." *Environmental Toxicology* 16 (2001): 523-34. Print.

Donati, Cosimo, Mary Drikas, Rob Hayes, and Gayle Newcombe. "Microcystin-LR Adsorption by Powdered Activated Carbon." *Water Research* 28.8 (1994): 1735-742. Print.

Edwards, Christine, Douglas Graham, Nicholas Fowler, and Linda A. Lawton. "Biodegradation of Microcystins and Nodularin in Freshwaters." *Chemosphere* 73.8 (2008): 1315-321. Print.

EPA Ohio (2015). Draft White Paper on Cyanotoxin Treatment.

EPA US (2014). Cyanobacteria and Cyanotoxins: Information for Drinking Water Systems.

EPA US (2015). Drinking Water Health Advisory for the Cyanobacterial Microcystin Toxins.

"Guidelines and Recommendations." US EPA, 15 Mar. 2016 Web. 05 Apr. 2016.

Ho, Lionel, Najwa Slyman, Uwe Kaeding, and Gayle Newcombe. "Optimizing PAC and Chlorination Practices for Cylindrospermopsin Removal." *American Water Works Association* 100.11 (2008): 88-96. *JSTOR*. Web. 15 Sept. 2015.

Ho, Lionel, Paul Lambling, Heriberto Bustamante, Phil Duker, and Gayle Newcombe. "Application of Powdered Activated Carbon for the Adsorption of Cylindrospermopsin and Microcystin Toxins from Drinking Water Supplies." *Water Research* 45.9 (2011): 2954-964. Web.

Huang, Winn-Jung, Bai-Ling Cheng, and Yung-ling Cheng. “Adsorption of microcystin-*LR* by three types of activated carbon.” *Journal of Hazardous Materials* 141 (2007): 115-122. Print.

Imanishi, Susumu, Hajime Kato, Masayoshi Mizuno, Kiyomi Tsuji, and Ken-Ichi Harada. "Bacterial Degradation of Microcystins and Nodularin." *Chemical Research in Toxicology* 18.3 (2005): 591-98. Print.

Julio, M. De, D. A. Fioravante, T. S. De Julio, F. I. Oroski, and N. J. D. Graham. "A Methodology for Optimising the Removal of Cyanobacteria Cells from a Brazilian Eutrophic Water." *Brazilian Journal of Chemical Engineering Braz. J. Chem. Eng.* 27.1 (2010): n. pag. Web.

Junfeng, Wang, Wu Pengfei, Chen Jian, and Yan Hai. "Biodegradation of Microcystin-RR by a New Isolated Sphingopyxis Sp. USTB-05." *Chinese Journal of Chemical Engineering* 18 (2010): 108-12. Print.

Lee, Jungju, and Harold W. Walker. "Effect of process variables and natural organic matter on removal of microcystin-LR by PAC-UF." *Environmental Science & Technology*. 40.23 (2006): 7336-7342. Print.

Lee, Jungju, and Harold W. Walker. "Adsorption of microcystin-Lr onto iron oxide nanoparticles." *Colloids and Surfaces A: Physiochemical and: Engineering Aspects*. 373 (2011): 94-100. Print.

"Microcystins." *On Cyanosite*. Biological Sciences at Purdue University and Wichita State University, n.d. Web. 16 Mar. 2015.

Peng, Lisa Y., and Jimmie L. Williams. Method of Making Mesoporous Carbon Using Pore Formers. Corning Incorporated, assignee. Patent 6024899. 15 Feb. 2000. Print.

Somdee, Theerasak, Michelle Thunders, John Ruck, Isabelle Lys, Margaret Allison, and Rachel Page. "Degradation of [Dha^7]MC-LR by a Microcystin Degrading Bacterium Isolated from Lake Rotoiti, New Zealand." *ISRN Microbiology* (2013): 1-8. Web. Article ID 596429

Sorilini, Sabrina, and C. Collivignarelli. “Microcystin-LR removal from drinking water supplies by chemical oxidation and activated carbon adsorption.” *Journal of Water Supply: Research and Technology-AQUA*. 60.7 (2011): 403-411. Print.

Szlag, David, James Sinclair, Benjamin Southwell, and Judy Westrick. "Cyanobacteria and Cyanotoxins Occurrence and Removal from Five High-Risk Conventional Treatment Drinking Water Plants." *Toxins* 7.6 (2015): 2198-220. Web.

Westrick, Judy A., David C. Szlag, Benjamin J. Southwell, and James Sinclair. "A Review of Cyanobacteria and Cyanotoxins Removal/inactivation in Drinking Water Treatment." *Analytical and Bioanalytical Chemistry* 397.5 (2010): 1705-714. Web.

Yan, Hai, Huasheng Wang, Junfeng Wang, Chunhua Yin, Song Ma, Xiaolu Liu, and Xueyao Yin. "Cloning and Expression of the First Gene for Biodegrading Microcystin LR by Sphingopyxis Sp. USTB-05." *Journal of Environmental Sciences* 24.10 (2012): 1816-822. Print.